

## ORIGINAL CONTRIBUTION

# Emergence of a New *Vibrio parahaemolyticus* Serotype in Raw Oysters

## A Prevention Quandary

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**V**IBRIO PARAHAEVOLYTICUS infection is usually associated with eating raw or undercooked shellfish, particularly oysters. Although *V. parahaemolyticus* is the leading cause of *Vibrio*-associated gastroenteritis in the United States,<sup>1,2</sup> outbreaks have rarely been reported before 1997.<sup>3,4</sup> In Asia, particularly in Japan and Taiwan, *V. parahaemolyticus* infections are commonly reported. Many recent *V. parahaemolyticus* infections in Asia have been caused by serotype O3:K6.<sup>5</sup> The outbreak described herein, which was caused by contaminated raw oysters ingested during late May, June, and early July 1998, was one of the largest *V. parahaemolyticus* outbreaks ever reported.

Strategies in the United States to prevent illness caused by contaminated shellfish include water quality monitoring and specific bacteriologic monitoring.

**Context** In May and June 1998, reported *Vibrio parahaemolyticus* infections increased sharply in Texas.

**Objective** To determine factors that contributed to the increase in *V. parahaemolyticus* infections.

**Design, Setting, and Participants** Cross-sectional survey of persons reporting gastroenteritis after eating seafood in Texas; survey of environmental conditions in Galveston Bay.

**Main Outcome Measures** Traceback of oysters, water quality measures in harvest areas, presence of *V. parahaemolyticus* in stool cultures; comparison of median values for environmental conditions before and during the outbreak compared with during the previous 5 years.

**Results** Between May 31 and July 10, 1998, 416 persons in 13 states reported having gastroenteritis after eating oysters harvested from Galveston Bay. All 28 available stool specimens from affected persons yielded *V. parahaemolyticus* serotype O3:K6 isolates. Oyster beds met current bacteriologic standards during harvest and fecal coliform counts in water samples were within acceptable limits. Median water temperature and salinity during May and June 1998 were 30.0°C and 29.6 parts per thousand (ppt) compared with 28.9°C and 15.6 ppt for the previous 5 years ( $P < .001$ ).

**Conclusions** This is the first reported outbreak of *V. parahaemolyticus* serotype O3:K6 infection in the United States. The emergence of a virulent serotype and elevated seawater temperatures and salinity levels may have contributed to this large multistate outbreak of *V. parahaemolyticus*. Bacteriologic monitoring at harvest sites did not prevent this outbreak, suggesting that current policy and regulations regarding the safety of raw oysters require reevaluation. Consumers and physicians should understand that raw or undercooked oysters can cause illness even if harvested from monitored beds. In patients who develop acute gastroenteritis within 4 days of consuming raw or undercooked oysters, a stool specimen should be tested for *Vibrio* species using specific media.

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ing. In 1975, following several food-borne disease outbreaks, including typhoid fever, that were caused by contaminated shellfish, the National Shellfish Sanitation Program created the Interstate Shellfish Sanitation Conference to improve the safety of shellfish. The Interstate Shellfish Sanitation Conference then instituted regulations requiring monitoring of water quality at oyster har-

vest sites for fecal coliforms to detect contamination with human sewage.

Although such monitoring may be useful for the prevention of typhoid fe-

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ver, it is not helpful for the prevention of *Vibrio* infections because *V. parahaemolyticus* and other *Vibrio* species are natural inhabitants of coastal waters globally, and their presence or absence is independent of human sewage. Regulations of the US Food and Drug Administration therefore require additional specific bacteriologic monitoring for *V. parahaemolyticus* in shellfish, with a requirement that shellfish have less than 10000 *V. parahaemolyticus* organisms per gram of meat. In several recent instances, however, outbreaks occurred even though no oyster samples yielded more than 10000 organisms per gram.<sup>3,4</sup> These outbreaks highlight the inadequacy of this strategy to protect public health.

## METHODS

### Outbreak

In mid-June 1998, the Texas Department of Health was notified of several apparent outbreaks of acute gastroenteritis among patrons of several seafood restaurants. When interviewed, those patrons reported eating raw oysters before becoming ill. Stool cultures from ill patrons yielded *V. parahaemolyticus*. Traceback of oysters eaten by affected persons indicated that the oysters were harvested from Galveston Bay. The Texas Department of Health notified the public of the outbreak and established a toll-free telephone hotline for the public to report acute gastroenteritis following shellfish consumption.

### Epidemiologic Investigation

Ill persons who called the hotline or a health department were interviewed using a standardized questionnaire concerning clinical history and symptoms and seafood consumption. If the affected person ate seafood at a restaurant, their dining companions were identified. We randomly selected 2 restaurant cohorts for intensive investigation from the list of reported events in which at least 10 people had eaten at the restaurant and at least 1 had become ill. Attendees of these events were interviewed about food items eaten and

possible subsequent gastrointestinal symptoms. Data from the 2 restaurant cohorts were combined and analyzed as 1 cohort because they had similar seafood exposures. For this cohort analysis, a case was defined as onset of diarrhea ( $\geq 3$  loose stools during a 24-hour period) within 24 hours of the event.

### Environmental Investigation

Oysters eaten by ill persons were traced to their respective harvest site by means of tags and dealer records. Oyster harvesters were interviewed and their records, including refrigeration records, were reviewed. The Texas Department of Health Seafood Safety Division operates 76 environmental monitoring stations in the oyster harvesting areas in Galveston Bay. Water temperature, salinity, and fecal coliform levels are routinely recorded weekly to monthly. For a historical comparison, data collected before and during the outbreak were compared with data collected during the previous 5 years.

### Laboratory Investigation

Clinical laboratories performed primary isolation of *V. parahaemolyticus* from human blood or stool specimens. Isolates were forwarded to the Texas Department of Health for confirmation and molecular subtyping, including pulsed-field gel electrophoresis (PFGE).<sup>6</sup> *V. parahaemolyticus* isolates were compared by PFGE with isolates collected from persons affected by recent outbreaks in Asia. Genomic DNA of *V. parahaemolyticus* was digested using 1 restriction enzyme (NotI). Isolates were serotyped<sup>7</sup> and tested for virulence markers (thermostable direct hemolysin and thermostable direct-related hemolysin genes) using polymerase chain reaction.<sup>8</sup>

Oyster samples collected between June and August 1998 from Galveston Bay were processed for *V. parahaemolyticus* bacterial counts and enumerated using the multiple-tube fermentation method. Samples consisted of 10 to 12 oysters (about 100 g) that were collected from 42 harvest sites.

The broth used for multiple-tube fermentation was alkaline peptone water and the suspected cultures were streaked onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar. Enumerated bacterial counts were reported using the most probable number (MPN) index. *V. parahaemolyticus* isolates from humans and oysters were also compared by PFGE.

### Statistical Analysis

Data were entered and analyzed using Epi Info Version 6.02 software (Centers for Disease Control and Prevention, Atlanta, Ga) and SAS Version 6.12 (SAS Institute Inc, Cary, NC). The Wilcoxon test was used to compare medians, and interquartile ranges (IQRs) were generated for environmental data. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated for raw oyster exposure in the cohort. Statistical significance was defined as  $P < .05$ .

## RESULTS

### Outbreak

There were 296 Texas residents who reported onset of diarrhea within 24 hours of eating seafood between May 31 and July 10 (FIGURE). The median age of affected persons was 42 years; 59% were male; 93% reported eating raw oysters before their onset of gastrointestinal illness; and 98% reported eating raw or undercooked oysters from either a restaurant or an oyster bar. Symptoms in addition to diarrhea included abdominal cramping (92%), nausea (66%), headache (51%), fever (46%), vomiting (42%), and bloody diarrhea (9%). The median duration of illness was 5 days (range, 1-30 days). A 51-year-old man whose illness lasted 30 days was hospitalized with bloody diarrhea. Fifteen persons were hospitalized and no deaths were reported. Three hospitalized patients who had eaten raw oysters were excluded from the case count because their incubation periods were longer than 24 hours.

An additional 120 *V. parahaemolyticus* infections associated with Galveston Bay oysters were reported from 12 other states (California, Florida, Geor-

gia, Oklahoma, Tennessee, Colorado, Virginia, Alabama, Kentucky, Massachusetts, New Jersey, and Missouri), with dates of illness onset of June 7 through July 4, 1998.

### Epidemiologic Investigation

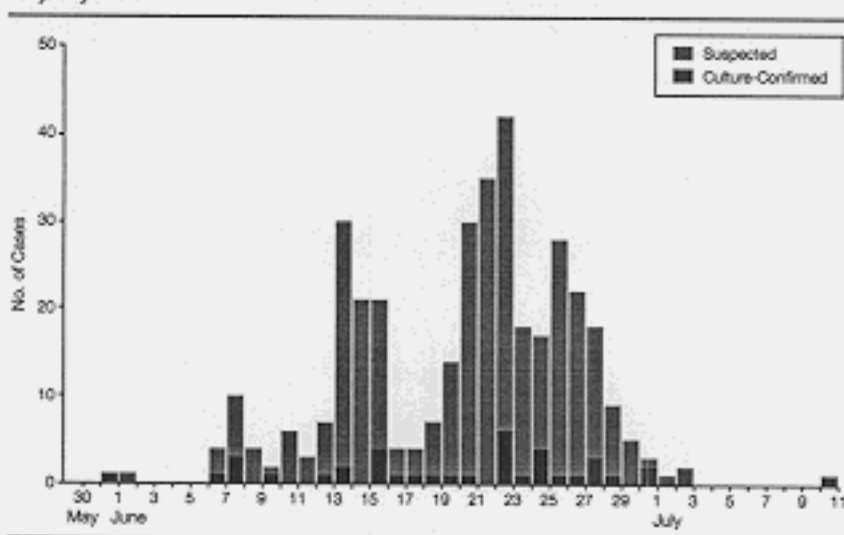
We interviewed 29 (64%) of the 45 persons in the 2 restaurant cohorts. Nine (75%) of the 12 respondents who consumed raw oysters became ill compared with none of the 17 respondents who had not eaten raw oysters (RR, undefined;  $P < .001$ ). The median number of oysters eaten by both ill and well persons in these cohorts was 5; 1 person became ill after consuming only 1 oyster. Eight (89%) of 9 persons who became ill reported having no underlying health conditions.

### Environmental Investigation

Traceback information was obtained for oysters that were consumed by 101 ill persons in Texas and other states. These harvest sites were widely distributed throughout Galveston Bay. The median time from harvest to refrigeration was 5.5 hours (range, 1.5–11.2 hours). Texas regulation allows for a maximum time of 10 hours between oyster harvest to refrigeration. Oysters were distributed through a complex system to retailers in at least 13 states. Harvesters sold approximately 1.5 million oysters from Galveston Bay during the outbreak period. Since the median number of oysters eaten during this investigation was 5 and the attack rate was 75%, we estimate that up to 300 000 persons may have been exposed and more than 200 000 persons may have become ill.

Environmental data at the 7 selected monitoring sites in Galveston Bay showed significantly higher median water temperature and salinity during May and June 1998 compared with data from the 5 previous years for the same months. In 1998, measurements were 30°C (IQR, 27.2°C–30.0°C) and 29.6 parts per thousand (ppt) (IQR, 15.2–29.6 ppt) compared with 28.9°C (IQR, 22.7°C–30.0°C) and 15.6 ppt (IQR, 1.3–17.0 ppt) for the previous 5 years

**Figure.** *Vibrio parahaemolyticus* Infections Reported in Texas, by Date of Illness Onset, May–July 1998



( $P < .001$ ). Fecal coliform counts were within regulatory limits before, during, and after the implicated oysters were harvested.

### Laboratory Results

There were 37 culture-confirmed *V. parahaemolyticus* infections (including 1 bloodstream infection) in Texas and 78 culture-confirmed infections reported from 12 other states. Each of the 28 clinical isolates tested at the Centers for Disease Control and Prevention were *V. parahaemolyticus* serotype O3:K6. These isolates were found to possess the thermostable direct hemolysin gene using polymerase chain reaction. Thirty-one (97%) of the 32 clinical *V. parahaemolyticus* isolates from patients in Texas were indistinguishable from each other by PFGE but were distinguishable from other historical *V. parahaemolyticus* strains. Galveston Bay and Asian *V. parahaemolyticus* O3:K6 strains showed distinct but closely related patterns (differing by 1 or 2 bands) by PFGE.<sup>9</sup>

Oysters from harvest sites contained *V. parahaemolyticus* at a median MPN of 15 organisms per gram of oyster meat (range, 3–4600) in the weeks following the outbreak. These oyster isolates had multiple PFGE patterns, but none of the oyster isolates har-

vested from sites in Galveston Bay had a PFGE pattern that matched the outbreak PFGE pattern.

### COMMENT

This large multistate outbreak of *V. parahaemolyticus* infections was associated with consumption of raw oysters harvested from approved beds in Galveston Bay, which were in compliance with current shellfish regulations. Current National Shellfish Sanitation Program monitoring of oyster beds uses fecal coliform testing,<sup>10</sup> which is irrelevant for detecting *Vibrio*<sup>11,12</sup> species and insensitive as an indicator for enteric viruses.<sup>13,14</sup> Since the infective dose of *V. parahaemolyticus* has been thought to be  $10^5$  to  $10^7$  viable cells ingested, the United States allows the sale of oysters if there are less than 10 000 MPN of *V. parahaemolyticus* per gram of oyster meat. However, during the weeks immediately following the outbreak, the median MPN of organisms found was 15 per gram of oyster meat, considerably lower than the 10 000-MPN/g threshold. Current policy regarding the safety of raw oysters requires reevaluation. It is possible that *V. parahaemolyticus* levels may have been higher during the actual outbreak period, although climatic conditions were similar and it seems unlikely that bacterial counts

would have changed significantly over such a short period.

### Clinical Features, Diagnosis, and Treatment

The clinical features of illness observed during this outbreak were generally consistent with those of previously reported *V. parahaemolyticus* gastroenteritis infections: a median incubation period of 17 hours (range, 4-90 hours) and a median duration of illness of 6 days have been reported.<sup>15</sup> Nevertheless, the fact that 15 patients in Texas were hospitalized for severe dehydration or bloody diarrhea suggests that only the most severe cases came to public health attention, which may have caused a reporting bias toward more severe cases. The high illness rate observed among exposed persons in the restaurant cohorts in this study (75% compared with a median illness rate of 56% in other *V. parahaemolyticus* outbreaks)<sup>15</sup> is a distinct feature of this outbreak and suggests that *V. parahaemolyticus* O3:K6 is more efficient in causing illness.

Transmission of *Vibrio* infections occurs primarily through consumption of raw or undercooked shellfish or by exposure of wounds to warm seawater.<sup>2</sup> The most common clinical presentation of *Vibrio* infection is self-limited gastroenteritis (59%), but wound infections (34%), primary septicemia (5%), and other infection sites (2%) may also occur with these organisms.<sup>15</sup> Persons who are immunocompromised or who have liver disease are at particular high risk for severe *Vibrio* infections and should be warned to avoid consumption of raw or undercooked shellfish.<sup>16</sup>

In patients who develop acute gastroenteritis within 4 days of consuming raw or undercooked shellfish, a stool specimen should be tested for *Vibrio* species using specific media such as TCBS agar. Isolation of *Vibrio* from stool is greatly enhanced through use of TCBS,<sup>17</sup> a selective medium for culturing *Vibrio*.

*Vibrio parahaemolyticus* strains that cause gastroenteritis are usually susceptible to antimicrobial agents routinely

used to treat enteric infections, although most cases of gastroenteritis can effectively be treated with oral rehydration alone. Wound and septicemia cases, however, should be treated similarly to *Vibrio vulnificus* infections, with ceftazidime and doxycycline or doxycycline in combination with ciprofloxacin or an aminoglycoside.

### Environmental Factors

Before this outbreak, *V. parahaemolyticus* serotype O3:K6 had not been previously reported in the United States. It appears that O3:K6 has established an ecological niche in Asia.<sup>3</sup> The O3:K6 clone has been described as similar to other pathogenic *V. parahaemolyticus* strains with regard to known virulence factors and antimicrobial susceptibility.<sup>18</sup> Although it has been suggested that the new clone may be better at persisting in the environment and establishing infection,<sup>19</sup> further studies are needed to confirm these hypotheses. It is also noteworthy that all of the cases identified during this outbreak were serotype O3:K6, but none of the oysters from a large environmental sample matched the outbreak strain using PFGE. In previous surveys, more than 95% of *V. parahaemolyticus* strains isolated from stool cultures had identified virulence factors, while less than 1% of strains from the environmental sample had these factors.<sup>20</sup> The reason for this discrepancy in virulence between human and environmental isolates is unknown. However, these surveys have shown that the majority of *V. parahaemolyticus* found in the marine environment and in food is nonpathogenic, which may make it more difficult to isolate outbreak strains.

It is not known how the O3:K6 strain emerged in Galveston Bay. However, ballast water from cargo ships entering the Gulf of Mexico is thought to be responsible for the introduction of the Latin American epidemic strain *Vibrio cholerae* serogroup O1 in Gulf Coast waters in 1991.<sup>21</sup> After emergence of serotype O3:K6, unusually elevated water temperatures and salinity levels likely provided a favorable environment for mul-

tiplication and dissemination of the organism. Elevated seawater temperatures during El Niño years (including 1998) have been shown to influence the incidence of *V. cholerae*<sup>22</sup> and other diarrheal diseases<sup>23</sup> and may also explain this outbreak of *V. parahaemolyticus* infections. A review of *V. vulnificus* infections found that 89% of oysters that caused these infections were harvested in waters with a temperature warmer than 22°C.<sup>24</sup> Since *Vibrio* infections are seasonal, restriction of harvesting during warmer months may reduce infections.

Lack of continuous refrigeration of oysters from harvest to consumption also may have contributed to this outbreak. The doubling time of *V. parahaemolyticus* at ambient temperatures is as short as 8 to 9 minutes (one of the fastest growing times known for bacteria). Therefore, oysters contaminated with only a small number of *V. parahaemolyticus* organisms can reach an infectious dose in only a few hours. During this outbreak, the median time from harvest to refrigeration was 5.5 hours. All harvesting boats have to return to shore before they are able to ice or refrigerate oysters. Thus, oysters left on the decks of harvesting boats even for short periods at warm temperatures can lead to rapid proliferation of *V. parahaemolyticus* to infectious levels. Requiring that ice be available on harvesting boats for immediate cooling of oysters would reduce multiplication of *V. parahaemolyticus* in oysters and thereby prevent illness.

### Prevention

*Vibrio parahaemolyticus* prevention strategies should be based on environmental trigger points, sampling schemes, public education, and the use of new technologies (eg, pasteurization or irradiation) to reduce or eliminate contamination. Public health departments should continue their vigilance and enhance *Vibrio* surveillance by making *Vibrio* isolations and infections reportable. We encourage tracebacks on all oysters linked to human cases of *V. vulnificus* or *V. parahaemolyticus* infection and specific linkage to environmental conditions in waters at the time of harvest.



In the meantime, consumers should thoroughly cook oysters and avoid eating raw or undercooked oysters, particularly in the warmer months. Warning labels on oyster products and restaurant menus may help educate consumers. Through a combination of all these efforts, further outbreaks may be prevented.

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**Disclaimer:** This work is in compliance with the guidelines of the US Department of Health and Human Services.

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**CORRECTIONS**

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**Incorrect Word:** In the Grand Rounds entitled "Clinical Features of and Recent Advances in Therapy for Fabry Disease" published in the December 6, 2000, issue of *THE JOURNAL* (2000;284:2771-2775), a word was changed incorrectly. On page 2774, in the third column, middle of the first paragraph, the sentence that begins "Mean insulin clearance" should have read "Mean inulin clearance."

To write an article of any sort is, to some extent, to reveal ourselves. Hence, even a medical article is, in a sense, something of an autobiography.

—J. Chalmers Da Costa (1863-1933)